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entitled Current Protocols in Molecular Biology, which are incorporated herein by reference) and chemical methods.

Throughout this specification the word "comprise", or variations such as "comprises" or

5 "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

### *ErbB2 crystals and crystal structures*

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The present invention provides a crystal comprising an ErbB2 polypeptide. Such crystals preferably are of the space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> with unit cell dimensions of a=75.96 Å, b=82.24 Å, and c=110.06 Å.

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As used herein, the term "crystal" means a structure (such as a three dimensional (3D) solid aggregate) in which the plane faces intersect at definite angles and in which there is a regular structure (such as internal structure) of the constituent chemical species. Thus, the term "crystal" can include any one of: a solid physical crystal form such as an experimentally prepared crystal, a 3D model based on the crystal structure, a 20 representation thereof such as a schematic representation thereof or a diagrammatic representation thereof, a data set thereof for a computer.

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Crystals according to the invention may be prepared using full-length ErbB2 polypeptides. However, preferably the extracellular domain is employed in isolation.

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Thus, preferably the ErbB2 polypeptide is a truncated polypeptide containing the extracellular domain and lacking the transmembrane domain and the intracellular tyrosine kinase domain. Typically, the extracellular domain comprises residues 1 to 632 (mature receptor numbering) of human ErbB2, or the equivalent thereof, or a truncated version thereof, preferably comprising amino acids 1 to 509, or the equivalent residues 30 in other ErbB2 polypeptides.

In a preferred embodiment the ErbB2 polypeptide is human ErbB2 (Accession No. A24571 – mature protein begins at residue 22). However, the ErbB2 polypeptide may also be obtained from other species, such as other mammalian species.

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crystallising the purified protein(s). Preferably the ErbB2 polypeptide contains the extracellular domain (amino acids 1 to 632 of the mature human polypeptide or a truncated version thereof, preferably comprising amino acids 1 to 509, or the equivalent residues in other ErbB2 polypeptides) but lacks the transmembrane and intracellular domains. Preferred host cells are those that provide for reduced glycosylation of recombinant polypeptides, such as a glycosylation-defective mammalian cell line e.g. the Lec8 Chinese hamster cell line, a derivative of CHO-K1 fibroblasts (ATCC CRC:1737) (Stanley, 1989, Mol. Cell Biol. 9: 377-383).

10 ErbB2 polypeptides may also be produced as fusion proteins, for example to aid in extraction and purification. Examples of fusion protein partners include glutathione-S-transferase (GST), hexahistidine, GAL4 (DNA binding and/or transcriptional activation domains) and beta-galactosidase. It may also be convenient to include a proteolytic cleavage site between the fusion protein partner and the protein sequence of interest to 15 allow removal of fusion protein sequences.

After expression, the proteins may be purified and/or concentrated, for example by immobilised metal affinity chromatography, ion-exchange chromatography, and/or gel filtration.

20 The protein(s) may be crystallised using known techniques. Usually, in a crystallisation process, a crystallisation buffer is prepared with a lower concentration of a precipitating agent necessary for crystal formation. For crystal formation, the concentration of the precipitating agent has to be increased, by addition of precipitating agent or by 25 diffusion of the precipitating agent between the crystallisation buffer and a reservoir buffer. Diffusion may be achieved by known techniques such as the "hanging drop" or the "sitting drop" method. In these methods, a drop of crystallisation buffer containing the protein (s) is hanging above or sitting beside a much larger pool of reservoir buffer. Alternatively, the balancing of the precipitating agent can be achieved through a semi- 30 permeable membrane that separates the crystallisation buffer and prevents dilution of the protein into the reservoir buffer.

We have found that the inclusion of about 15% PEG 1500 provides optimal crystallization conditions for the extracellular domain of human ErbB2.